

**The Chemistry of Cigar Smoke. II.
Some Components of the
Neutral Fraction**

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Introduction

The smoke from cigars consists of a complex mixture of gaseous and particulate components which, for the most part, have not yet been identified. Although a large number of chemical constituents have been isolated in the many recent studies on cigarette smoke, work on cigar smoke has not kept pace. To date, only 33 chemical constituents of cigar smoke have been identified and described in some 43 publications.

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As expected, nicotine has been of prime interest in cigar smoke studies, particularly along quantitative lines. Several investigators reported the presence of certain polycyclic hydrocarbons in microgram quantities. These are anthracene, pyrene, fluoranthene, phenanthrene, acenaphthalene, 1, 2-benzanthracene, 1, 2-benzpyrene and 3, 4-benzpyrene. Among the aliphatic hydrocarbons, only methane and hentriacontane ($C_{31}H_{64}$) have been reported. Although Wenusch, Abeles and Paschke and Kissling had found higher hydrocarbons present, one of which may have been hentriacontane, they did not complete their identifications. This was left to

Schürch and Winterstein who found hentriacontane in concentrations as high as 5% in cigar smoke. The inorganic gaseous components that have been reported present in cigar smoke are oxygen, carbon monoxide, carbon dioxide, hydrogen cyanide, hydrogen sulfide, hydrogen thiocyanide and cyanogen. Among the non-nicotine bases reported are pyridine, pyrrole and ammonia.

The only neutral components thus far identified are formaldehyde, acetaldehyde, diacetyl and methanol. Schürch and Winterstein isolated, but were unable to identify, a ketone (possibly dipalmityl ketone) melting at $79^{\circ}C$, a phytosterol melting at $135^{\circ}C$, and an "odor compound" having the formula $C_{10}H_{14}O$ and boiling at $50-55^{\circ}C$.

Miscellaneous substances that have been reported by a variety of workers include: arsenic, scopoletin, resin acids, "reducing substances" and "tar".

The lack of knowledge in regard to the chemical composition of the smoke coupled with a profound interest in the flavor and odor of cigar smoke prompted the Cigar Manufacturers Association of America, in cooperation with the United States Department of Agriculture, to begin the present investigation. This report presents some of the initial findings of this work and concerns the isolation and identification of certain components of the neutral fraction of the smoke.

Experimental

The starting material for these

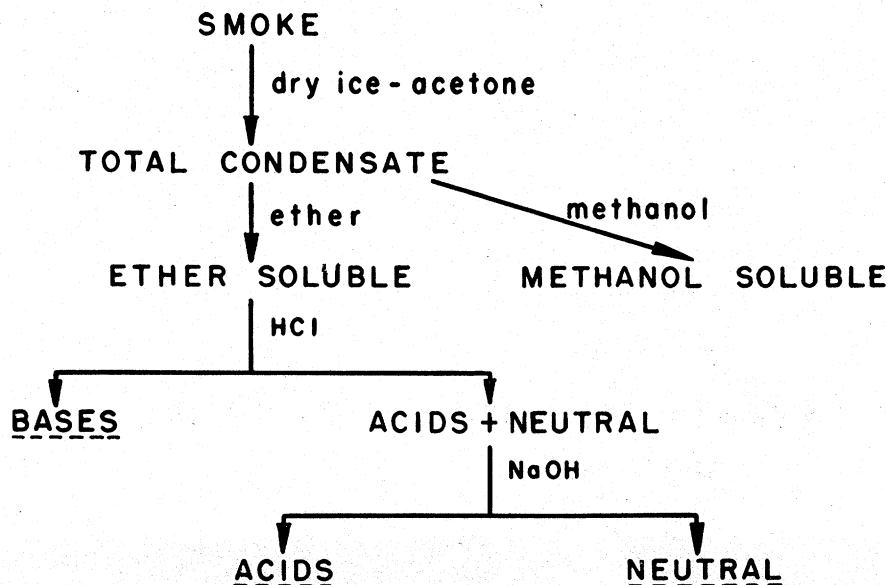


Figure 1. General fractionation scheme.

studies was obtained by the condensation of cigar smoke at low temperature. Cigars were smoked on a specially designed apparatus that has already been described. The smoking conditions were those generally used in cigarette smoking: rate, one puff per minute; duration of puff, two seconds; volume of puff, 35 ml.

The cigars were of the long Havana filler type, measuring 140 mm in length. Two thirds (93 mm) of this length was smoked, requiring an average of 65 puffs per cigar. The number of cigars smoked in these studies has varied from as few as two to as many as 100 in a group, depending upon the amount of condensate desired; however, the usual number has been 20. Daily checks on the timing cycles were made to assure a maximum average error of less than one per cent.

During the smoking of each group of cigars and the collection of condensates therefrom, the cold traps were maintained at -70°C by means of an adequate supply of dry ice and acetone. When sufficient condensate had been accumulated, the apparatus was dismantled, the traps removed, all joints freed of grease (petroleum jelly), and the condensate washed out with ethyl ether,³ then with methanol. The two solutions were stored separately in the freezer until used. This two-step rinsing procedure provided a preliminary fractionation into ether-soluble and methanol-soluble materials.

Since the ether-soluble part of the condensate comprised about 95% of the total, this portion was taken for further fractionation. By washing an ether solution of this material with 5% HCl, then with 5% NaOH, and reextracting the washings with ether, basic and acidic materials were separated, leaving a neutral fraction. This is illustrated in simplified form in Figure 1. Based on data from several groups of cigars, yields of these fractions per cigar were approximately: total condensate, 105 mg; ether-soluble, 100 mg; methanol-soluble, 5 mg; basic fraction, 25 mg; acidic fraction, 25 mg; neutral fraction, 50 mg.

In order to investigate the carbonyl components of the smoke, a neutral fraction obtained in this manner was reacted with 2, 4-dinitrophenylhydrazine. The resulting precipitate of mixed dinitrophenylhydrazones (DNPH's) was extracted with benzene to give two major fractions

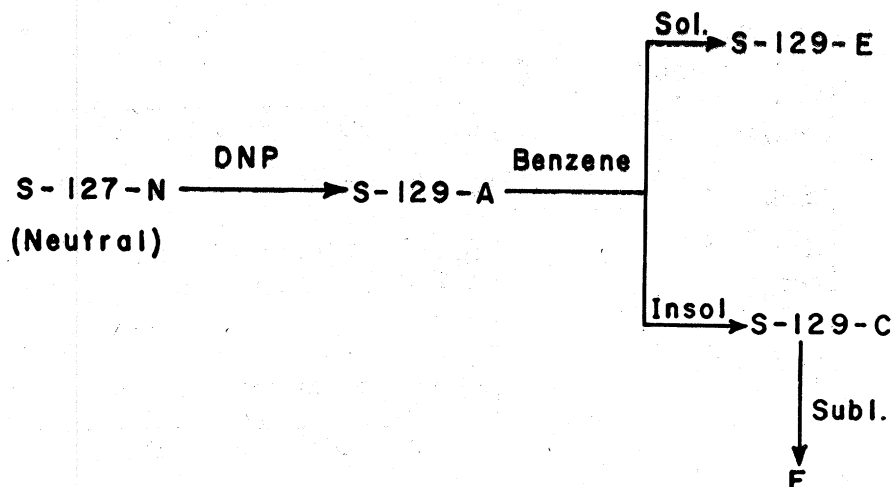


Figure 2. Fractionation of the neutral portion of the smoke condensate.

(S-129-E and S-129-C) as shown in Figure 2. These two fractions (or two obtained by identical procedures) were subjected to a number of further fractionations that resulted in the isolation and identification of several components of the smoke.

Formaldehyde—From S-129-C it was possible to isolate, by sublimation, approximately 0.005 mg⁴ of a compound F (Figure 2). This DNPH melted at $168.4\text{--}170^{\circ}\text{C}$ ⁵ and appeared to be the derivative of either formaldehyde or acetaldehyde. An examination of its ultraviolet absorption properties (Beckman DU Spectrophotometer) in ethanol and chloroform, in conjunction with authentic samples, gave the results shown in Table 1. From these data and other characteristics of the absorption curves, it was concluded that compound F was formaldehyde-2, 4-DNPH. This was further substantiated later in the finding of formaldehyde by paper chromatography.

Methyl Ethyl Ketone—Fraction S-129-C was chromatographed on a column (22 x 150 mm) composed of silicic acid and Filter-cel (2:1 by

weight). Development was conducted with petroleum ether and gradually increasing concentrations of ethyl ether. Four bands were obtained as shown in Figure 3. After elution, samples of these fractions were placed on a paper chromatogram. The multiplicity of spots obtained clearly showed that, with the possible exception of S-180-A, they were still mixtures (Figure 3). The paper chromatographic method used will be described in a later section of this report.

After evaporation of solvents, S-180-A consisted of orange crystals having a melting point of 84°C . This material was recrystallized (0.15 mg; m.p. 89°C) and an ultraviolet absorption spectrum obtained. The spectrum showed the compound to be a DNPH of a simple ketone. The structure of S-180-A was studied by infrared analysis (Perkin-Elmer Model 21) and comparisons were made with a series of known ketone derivatives. An almost perfect "fit" of the pattern of S-180-A was found with that of methyl ethyl ketone-2, 4-DNPH. This was confirmed by X-ray diffraction (General Electric XRD-3).

Samples of the isolated material and the authentic derivative gave identical R_f values when run on a paper chromatogram (see later section). Also shown on the chromatogram were several minor impurity

4. All yields are calculated on a basis of content per one cigar.

5. Melting points were taken on a modified Fisher-Johns block,⁶ using Anschütz thermometers and a polarizing microscope, and are uncorrected.

6. Mention of a specific commercial product does not constitute an endorsement by the United States Department of Agriculture over similar items not mentioned.

Table 1. Ultraviolet absorption maxima.

DNPH	Wavelengths of Maximum Absorption (mμ)	
	Ethanol	Chloroform
Compound F	350	347
Formaldehyde	348	346
Acetaldehyde	360	354

3. All solvents were of highest purity obtainable from commercial sources and were used without further purification. A test for carbonyls on all solvents showed this practice to be valid.

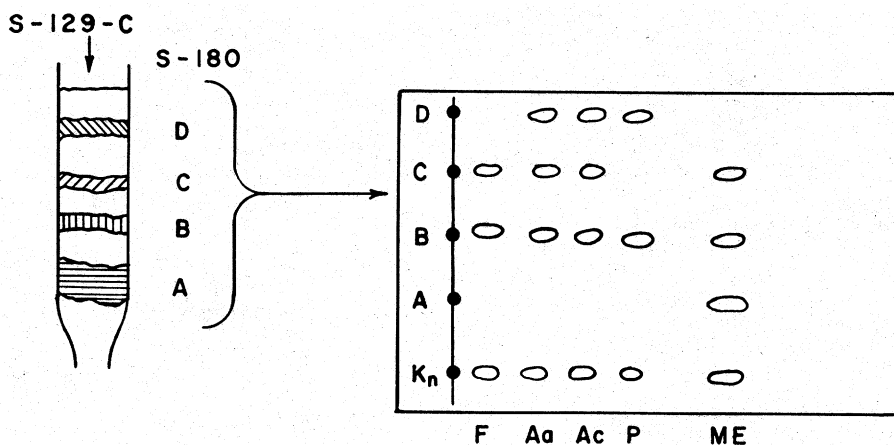


Figure 3. Chromatography of DNPH mixture S-129-C.

Legend: A, B, C and D = DNPH fractions; Kn = Known mixture of DNPH's; F = Formaldehyde-DNPH; Aa = Acetaldehyde-DNPH; Ac = Acetone-DNPH; P = Propionaldehyde-DNPH; ME = Methyl ethyl ketone-DNPH.

spots that separated from S-180-A. (These spots are not shown in Figure 3 for reasons of simplicity.) These impurities undoubtedly are responsible for the discrepancy in melting points (authentic methyl ethyl ketone-DNPH, m.p. 111° C).

A *Silicone* — Fraction S-129-E (Figure 2) was washed with 2N HCl to remove excess reagent and then chromatographed on a column of silicic acid and Filter-cel in the same manner as S-129-C above. Four bands were produced (figure 4). After elution, fraction S-141-A was precipitated and recrystallized until it yielded 0.95 mg of pale yellow material, m.p. 58.8-60° C. This product

failed to display any absorption maxima in the ultraviolet spectrum; hence, it was obviously not a DNPH. An infrared pattern (Figure 5, A) indicated the presence of silicon in an organic structure, probably as an oxysilane (silicone). A striking resemblance to the infrared pattern of a commercial silicone polymer (Figure 5, B) suggested possible contamination, since a silicone grease had been used on some of the joints of the glass apparatus involved in the processing of the smoke.

In order to determine whether the silicone had actually come from the cigars or been introduced by the

grease as a contaminant, the following experiments were conducted:

1. After carefully degreasing all glassware by treatment with a hot saturated solution of NaOH in ethanol and using only pure hydrocarbon grease (petroleum jelly instead of silicone), another group of 20 cigars was smoked. The condensates were processed exactly as before. The neutral fraction was again reacted with 2, 4-dinitrophenylhydrazine and a fraction (S-168-A) was separated from the mixed derivatives by chromatography. This material was shown by infrared analysis to contain a silicone similar to S-141-A. S-168-A had a crude weight of 5.0 mg, m.p. 58-60° C.
2. The presence of silicon in both S-141-A and S-168-A was confirmed by positive qualitative tests by the method of Feigl (9). Appropriate blanks were negative.
3. A complete blank-run through the entire isolation procedure served as a check on all reagents and solvents for the presence of silicones. The result was negative. A separate blank on the silicic acid column was negative. An infrared pattern on the 2, 4-dinitrophenylhydrazine reagent was also negative.
4. A blank-run on the air in the laboratory was conducted by pulling through the cold traps the air-equivalent of smoking 20 cigars. This amounted to 45.5 liters of air in 1300 puffs. A test for silicon in the condensate was negative. A similar blank was run while an open beaker of silicone oil was being heated and maintained at 200° C within four feet of the air intake of the smoking machine. The result was again negative.
5. As a check on the possible introduction of a silicone during manufacture, an extract was made by surface washing of the tobacco leaves from 20 cigars with organic solvents; no organic silicon was present. After grinding to break up the tissue cells and subsequent extraction, organic silicon was found in the filler, binder and wrapper tobacco.

From these experiments, it was concluded that the silicone found in the smoke was derived from the cigars and that the silicon was originally present in the cells of the tobacco leaf in an organic structure

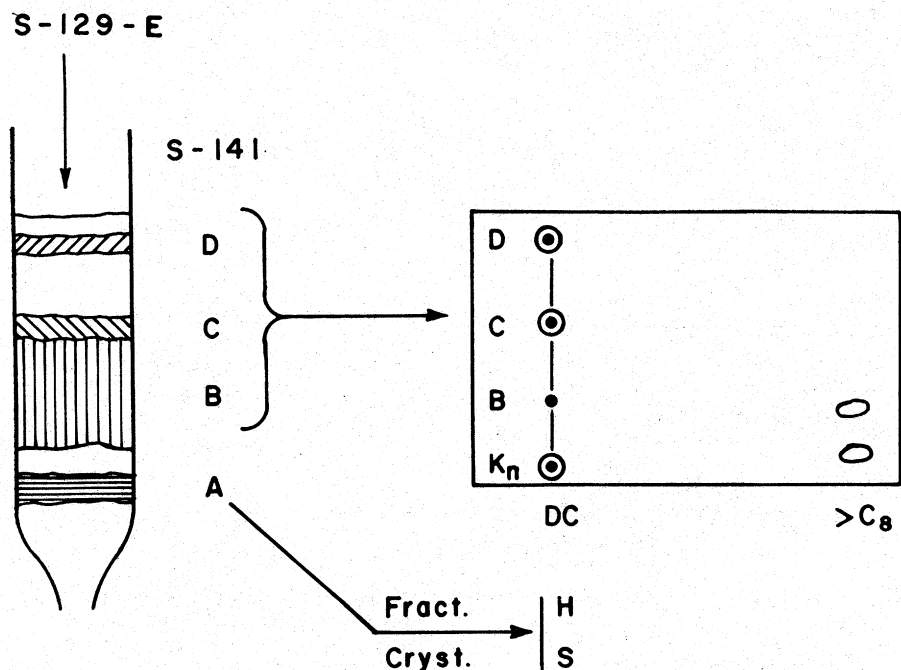


Figure 4. Chromatography of DNPH mixture S-129-E.

Legend: A, B, C and D = Column fractions; Kn = Known mixture of DNPH's; DC = Dicarboxyl-bis-DNPH; >C₈ = DNPH of a carbonyl of greater than eight carbons; H = Hentriacontane; S = Silicone.

f the same or of a precursor nature.

Fraction S-168-A was fractionally recrystallized to give a component, S-178-A, that contained only a trace of silicon, and a second fraction, S-178-B (0.17 mg, m.p. 34-36° C), that was the silicone. These findings were made by infrared absorption study as well as qualitative micro analysis.

Hentriacontane—While working on the above silicone-containing fraction, S-168-A, a non-silicon component, S-178-A, was separated. This material weighed 1.0 mg and melted at 62° C. By infrared pattern S-178-A was found to be a long-chain, saturated hydrocarbon. Upon further investigation this hydrocarbon was identified as hentriacontane, $C_{31}H_{64}$, exhibiting the comparative characteristics given in Table 2.

Other Aldehydes and Ketones—In addition to the formaldehyde and methyl ethyl ketone mentioned above, several other carbonyl components were identified by paper chromatography. The technique used was based on the work of Heulin, in which samples are run in an ascending system and separated by a methanol-heptane solvent mixture. The results are shown in Figures 3 and 4. Fractions S-180-A, B, C and D were chromatographed as illustrated in Figure 3. A mixture of known DNPH's (Kn) was also run, as indicated, for the purpose of identifying unknown spots. In like manner, Figure 4 illustrates the chromatography of S-141-B, C and D. In this case the known mixture consisted of dicarbonyl bis-DNPH's and a monocarbonyl-DNPH of C_9 or higher.

By means of this comparative type of chromatographic study, including the use of mixed samples, the following carbonyls were identified in the various fractions: formaldehyde, acetaldehyde, propionaldehyde, acetone, methyl ethyl ketone and a monocarbonyl of C_9 or higher.

The dicarbonyls that were observed in Figure 4 could not be identified because of lack of resolution in this system. The C_9 or higher monocarbonyl could not be further characterized because it moved with the solvent front.

Discussion

The finding of several simple aldehydes and ketones in cigar smoke was not unexpected. All of these have been reported present in the smoke from cigarettes and two of them in pipe tobacco smoke. However, only formaldehyde and acetaldehyde have been found previously in cigar smoke. Propionaldehyde, acetone and methyl ethyl

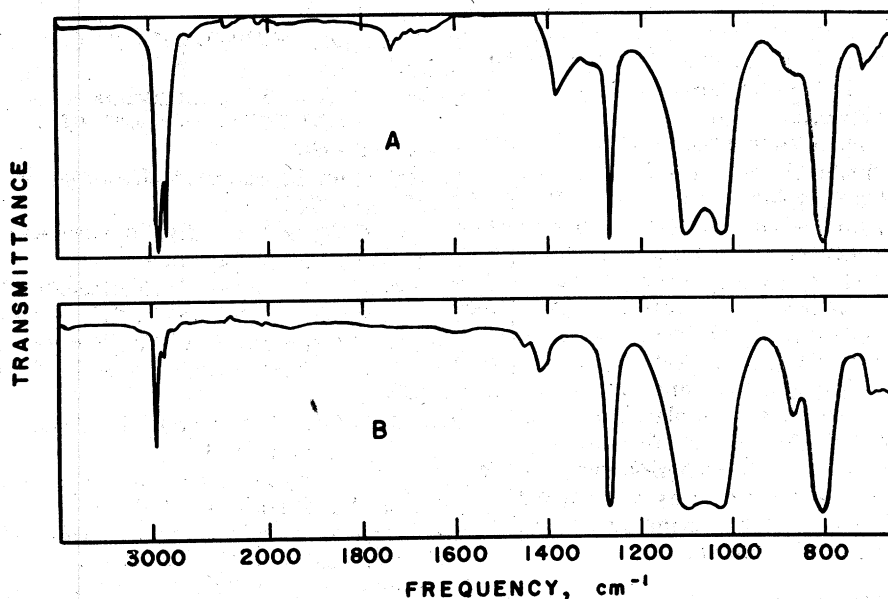


Figure 5. Infrared absorption patterns.
A. Silicone isolated from smoke.
B. A commercial silicone polymer.

Table 2. Comparison of S-178-A and authentic hentriacontane

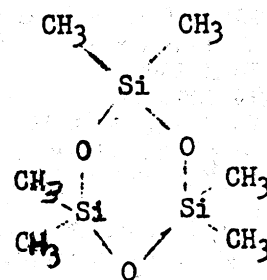
	S-178-A	Hentriacontane
Melting point	62° C	64° C
X-ray diffraction:		
Long spacing	42.6 ± 0.5	43.0
Side spacings	4.13 3.72	4.14 3.74

ketone are thus new findings for the latter type of smoke.

Hentriacontane has, of course, been found by many workers to be present in cigar and cigarette smoke. Its occurrence in a DNPH fraction can be explained by its similarity in solubility properties to the carbonyl derivatives.

The most surprising component found was the oxysilane, or silicone. Although there have been reports in the literature of silicon occurring in organic structures in plant and animal tissues, such reports have never included tobacco. Cogbill and Hobbs and Williams have found silicon in cigarette smoke only by spectrographic analysis. Thus the present report constitutes the first finding of an organo-silicon compound in tobacco smoke and (qualitatively) in tobacco. It would also appear to be the only isolation of a silicone derived from natural sources.

The exact structure of the silicone is yet unknown. From all data presently available, the most probable configuration is a ring composed of alternating silicon and oxygen atoms with hydrocarbon side chains on each silicon such as:



This compound and others higher in the series, $[(CH_3)_2SiO]_{3-8}$, have been synthesized and reported in the literature (14, 61, 64). Their physical properties and infrared spectra correspond well with those of the isolated material. From this comparison, and taking into account the properties and spectrum of the high molecular weight polymer, it would appear that the isolated silicone is a member of this series, probably in the range of $[(CH_3)_2SiO]_{10-50}$. This places it somewhere between the known lower members of the series and the high polymers. The occurrence of ethyl or propyl groups on the silicon atoms instead of the methyl groups is also possible. Further work is necessary to elucidate these points.

Summary

Cigars were smoked on a specially designed apparatus and the smoke collected by low temperature condensation. A neutral fraction of the smoke condensate was obtained by chemical separation. This fraction was explored for carbonyl components by means of a dinitrophenylhydrazine reaction and subsequent fractionation of the mixed derivatives. By a combination of chemical and chromatographic procedures in conjunction with certain physical techniques, five carbonyl components of the smoke were isolated and identified. These were: formaldehyde, acetaldehyde, propionaldehyde, acetone and methyl ethyl ketone. In addition, hentriacontane and a silicone were found.

Acknowledgement

This work was supported in part by funds made available by the Cigar Manufacturers Association of America, Incorporated.

The author expresses his thanks to Drs. C. R. Eddy and H. Susi and Mr. C. Leander for the infrared studies and to Mr. D. Lutz for the X-ray diffraction work.

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